

LBNL-60341 Poster

Toward a comprehensive overview of the *Bacillus cereus* group genomes.

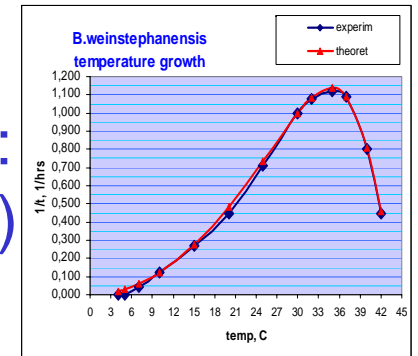
Alexei Sorokin¹, Sandrine Auger¹, Nathalie Galleron¹, S.Dusko Ehrlich¹, Eugene Goltsman², Paul Richardson², Alla Lapidus²

¹ *Génétique Microbienne, CRJ INRA, Jouy-en-Josas, France*

² *DOE Joint Genome Institute, Walnut Creek, CA, USA*

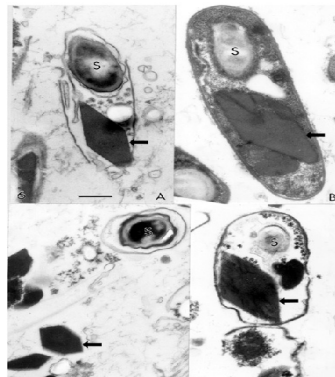
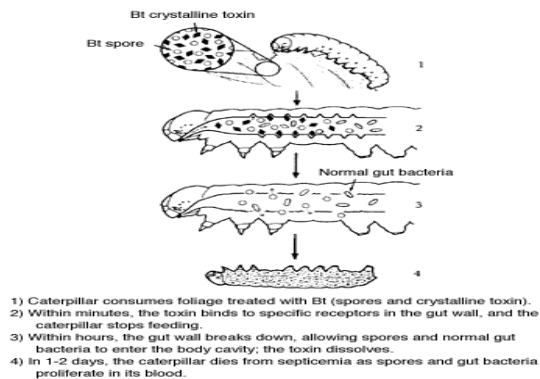
B. cereus group : four distinctive phenotypes

Bacillus
weihenstephanensis :
psychrotrophic (<7°C)



Bacillus thuringiensis :
world wide used bio-pesticide

Action of *Bacillus thuringiensis* var. *kurstaki* on caterpillars



Feng et al, World Journ Biotechnol
Microbiol, 2001, 17, 119-123



DiFranco et al, MMC
Microbiology, 2002, 2:33.

Bacillus mycoides :
form rhizoidal colonies



(after Peter C. B. Turnbull)

Bacillus anthracis :
etiological agent of Anthrax

The *Bacillus cereus* group consists of gram-positive, spore-forming bacteria with an impact on human activities due to their pathogenic properties. The most famous pathogens of animals and insects, *B. anthracis* and *B. thuringiensis*, carry their toxins in large plasmids. However, the bacteria of this group are also involved in mild food poisoning, causing rather noxious, although not fatal, vomiting or diarrhea. The emetic group strains appear to represent a very narrow phylogenetic group, and the corresponding toxin is encoded by a plasmid¹. Contrary to that, the characterized diarrheic toxins (Nhe, Hbl and CytK) are always present in the chromosome.

Three different strains were selected for complete sequencing, to provide the comprehensive genomic characterization of the group. The work is being done as a collaboration of INRA, JGI and Génoscope (France). The strain NVH398-91 is now completely sequenced. The strain was isolated from a severe food poisoning case in France². It contains a gene for only one of diarrheic toxins - CytK, which is highly expressed and effective in cell lysing tests^{3,4}. The strain differs from others of *B. cereus* group and has a compact chromosome of 4.1 Mb, compared to 5.2-5.4 Mb genomes of other sequenced strains. This strain is the first bacterium of the group in which a unique and highly effective toxin is chromosome-encoded. Divergence of the strain from others and its phenotypic particularities inspires to consider it as a new species, for which we propose a name *Bacillus cytotoxis*. However, only one such strain was described so far.

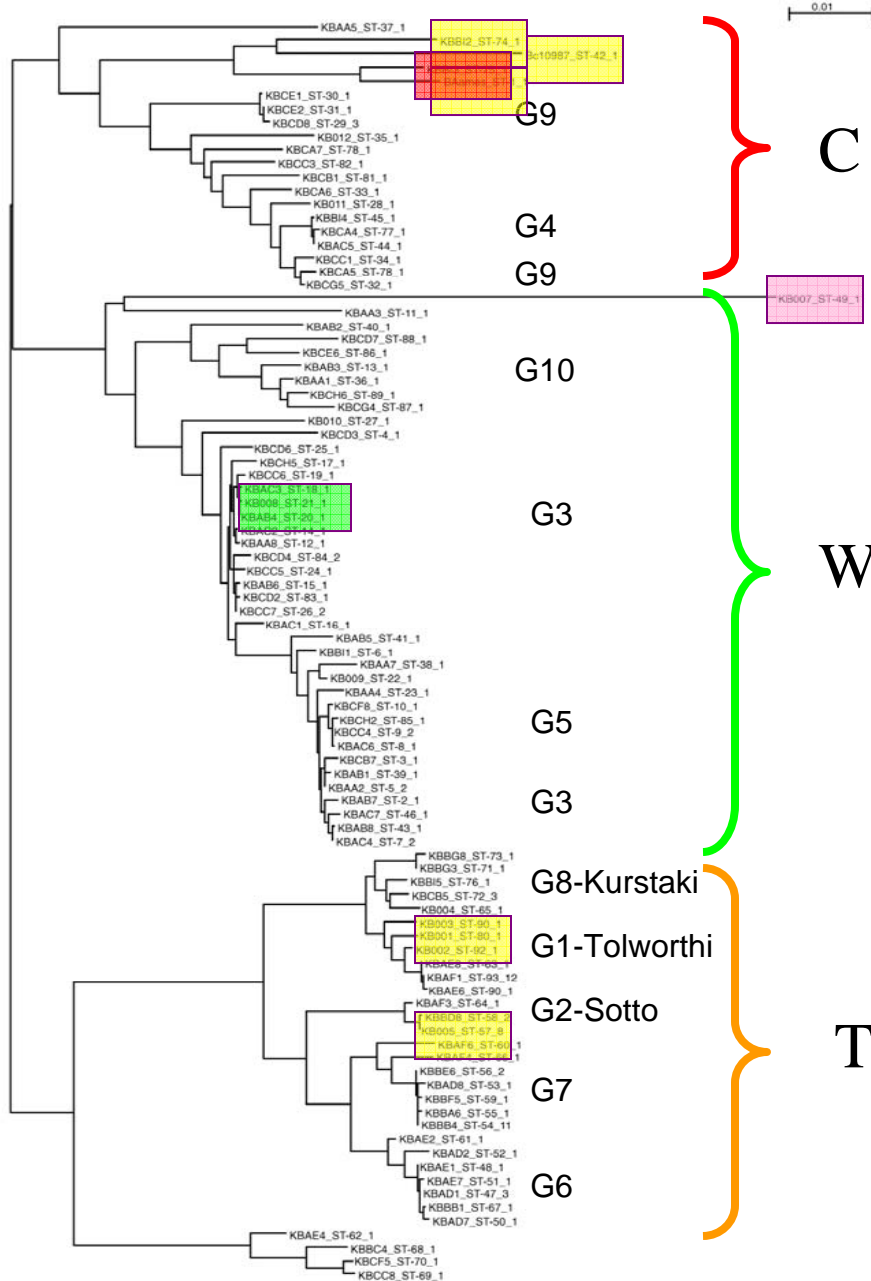
The second strain, KBAB4, isolated from forest soil near Versailles (France), is type representative of recently recognized species - *B. weihenstephanensis*^{5,6}. It contains a total of 5.86 Mb of genetic material, which includes genes for Nhe and Hbl, but lacks genes for CytK and HlyII. Although closely related strains are frequently reported to be food contaminants, they are usually not poisonous. However, the presence of Nhe and Hbl genes suggests that such strains can also become toxic.

Finally, the third strain, *B. cereus* F0837/76, can be regarded as one of typical strains that can cause a severe diarrheic food poisoning. It synthesizes at least two characteristic toxins, Nhe and Hbl, in elevated amounts and was isolated from a contaminated surgical wound in a hospital⁷⁻⁹. Genomically the strain appeared to be very closely related to *B. anthracis* and this is probably the closest to it *B. cereus* strain known. It does not however contain the relevant pathogenic plasmids.

Together with several other sequenced strains of the group¹⁰⁻¹² this work will provide a comprehensive overview of the genomic organisations in these important organisms. It will serve to better understand its pathogenic potential, psychrotrophy related features and the mechanisms of fast adaptation to pathogenicity related niches.

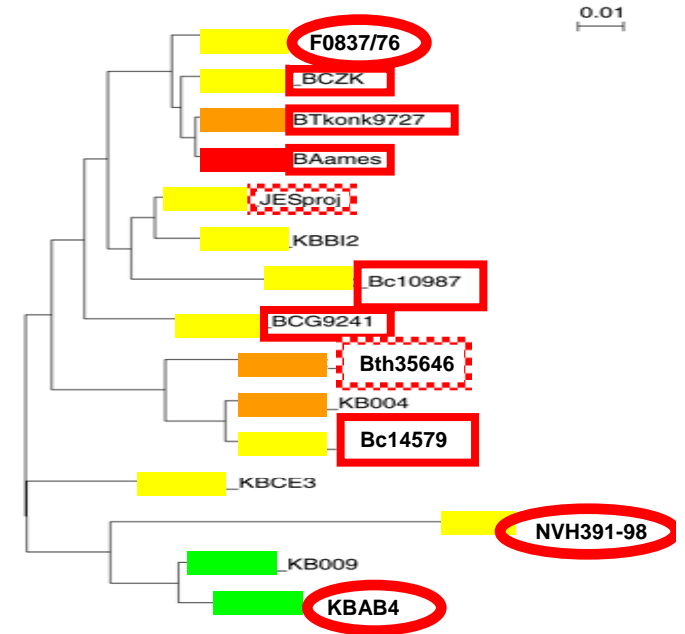
1. Ehling-Schulz et al, *Microbiology*, 2005, 151, 183-197.
2. Lund et al, *Mol Microbiol*, 2000, 38, 254-261.
3. Brillard and Lereclus, *Microbiology*, 2004, 150, 2699-2705.
4. Fagerlund et al, *Microbiology*, 2004, 150, 2689-2697.
5. Lechner et al, *Int J Syst Bacteriol*, 1998, 48, 1373-1382.
6. Sorokin et al, *Appl Env Microbiol*, 2006, 72, 1569-1578.
7. Turnbull et al, *Journ Clin Pathol*, 1979, 32, 289-293.
8. Beecher and MacMillan, *Infect Immun*, 1990, 58, 2220-2227.
9. Lund and Granum, *Microbiology*, 1997, 143, 3329-3336.
10. Ivanova et al, *Nature*, 2003, 423, 87-91.
11. Read et al, *Nature*, 2003, 423, 81-86.
12. Rasko et al, *Nucl Acids Res*, 2004, 32, 977-988.

B. cereus group phylogeny and genomic projects



Simplified tree, indicating completely or partially sequenced strains:

complete sequence
 this project
 unclear status



MLST based phylogenetic tree

three main strain clusters (C, T and W) were detected using concatenated sequences for six genes.

Sorokin et al, *Appl Env Microbiol*, 2006, 72, 1569

Genomic sequencing projects indicated by colours:

“French killer” (JGI) :

KB007 = NVH 391-98

Type psychrotroph (JGI) :

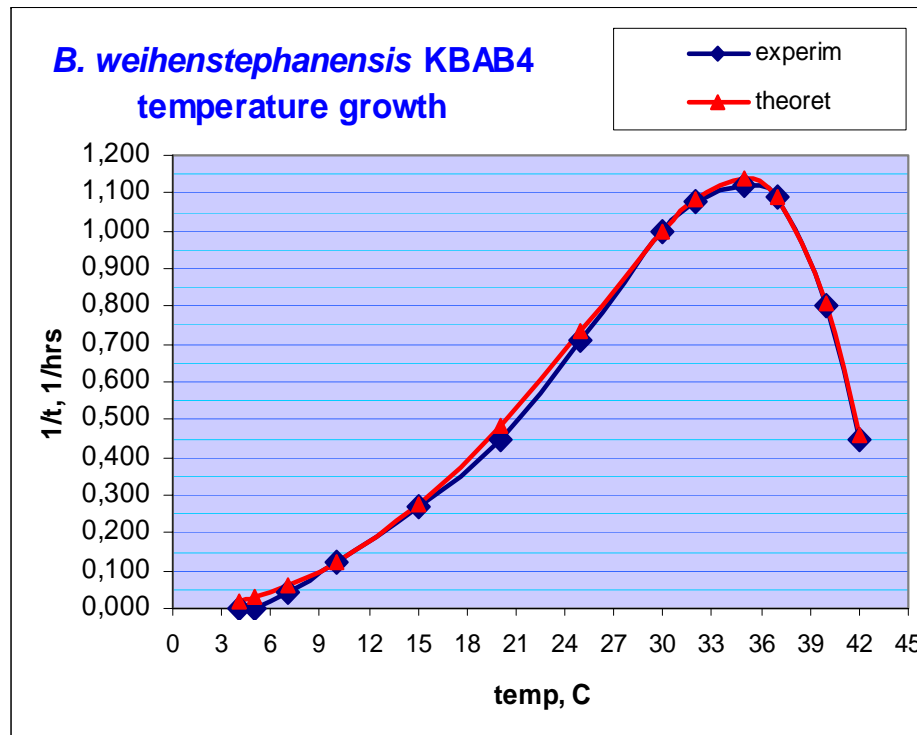
KBAB4 from Versailles Collection

“type diarrhoeic” (Génoscope) :

KBB13 = F0837/76

Other genomic projects (completed or not)

Psychrotrophic strains : growth at different temperatures

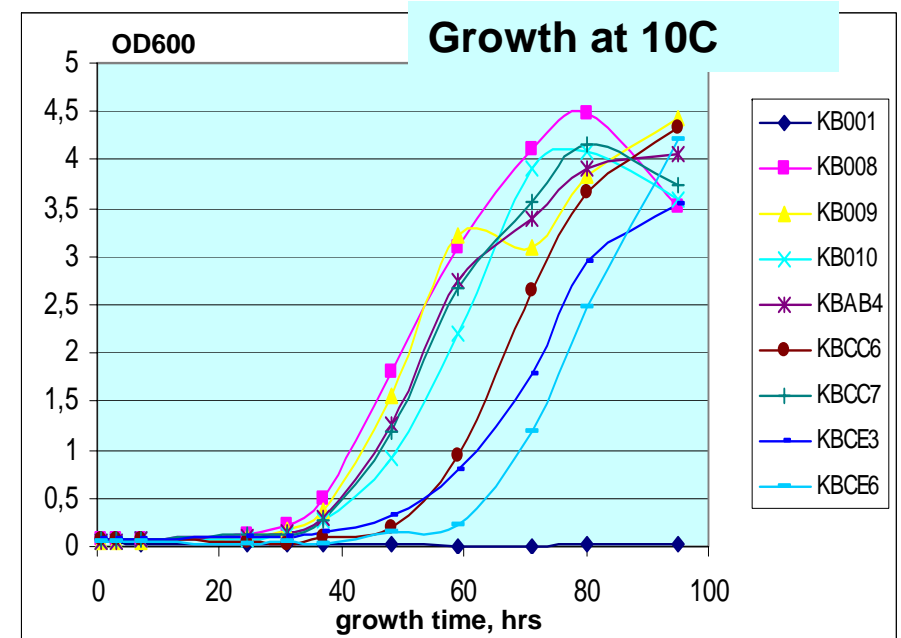


The cells of the strain *B. weihenstephanensis* KBAB4 were diluted 1:100 after overnight growth at 30°C and propagated under aeration at different temperatures. The reverse of the doubling time during the exponential growth is plotted against temperature. Experimental points in the low temperature region were measured in the range 8 to 30°C, then interpolated and extrapolated using the model of Ratkowsky et al (top right).

$$k = A * \exp(-E/RT)$$

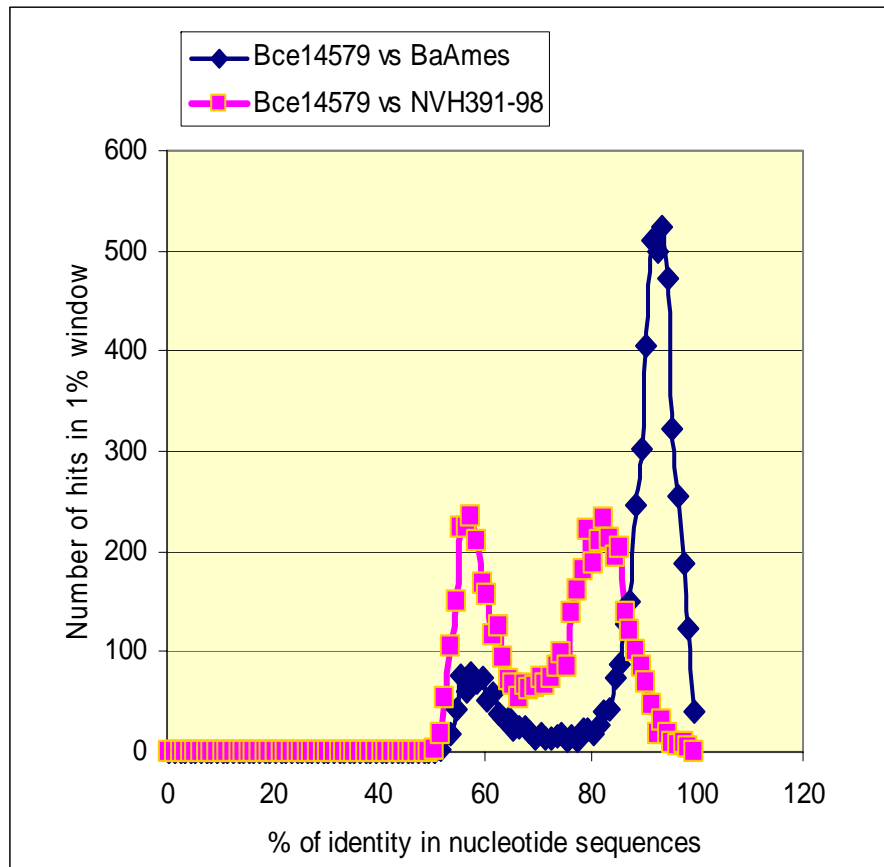
$$\sqrt{\mu} = b * (T - T_{\min}) * \{1 - \exp[c(T - T_{\max})]\}$$

(Ratkowsky et al, 1982)

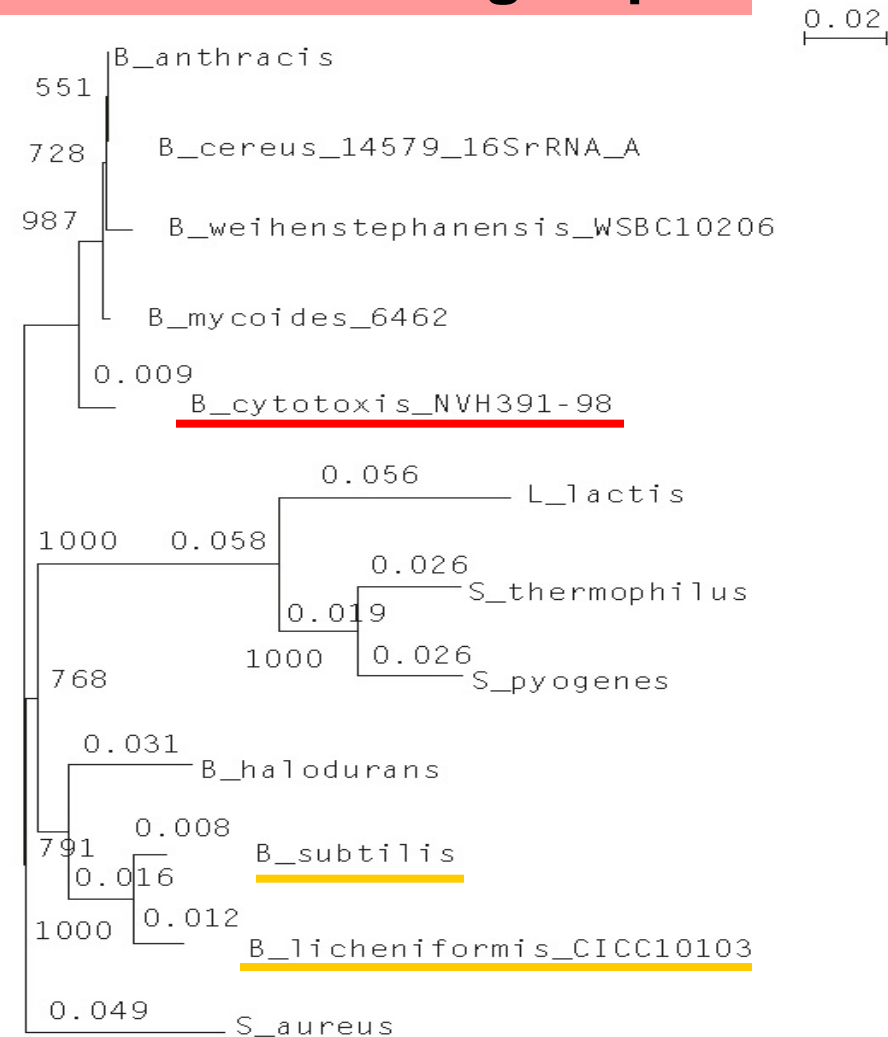


The cells of different strains of the *B. cereus* group were diluted 1:100 after overnight growth at 30°C and propagated at 10°C. Strain KB001 is mesophilic, strains KBCC7, CE3 and CE6 have intermediate psychrotrophic growth status. Other strains are psychrotrophic.

Is *B. cytotoxis* a new species in the *B. cereus* group?

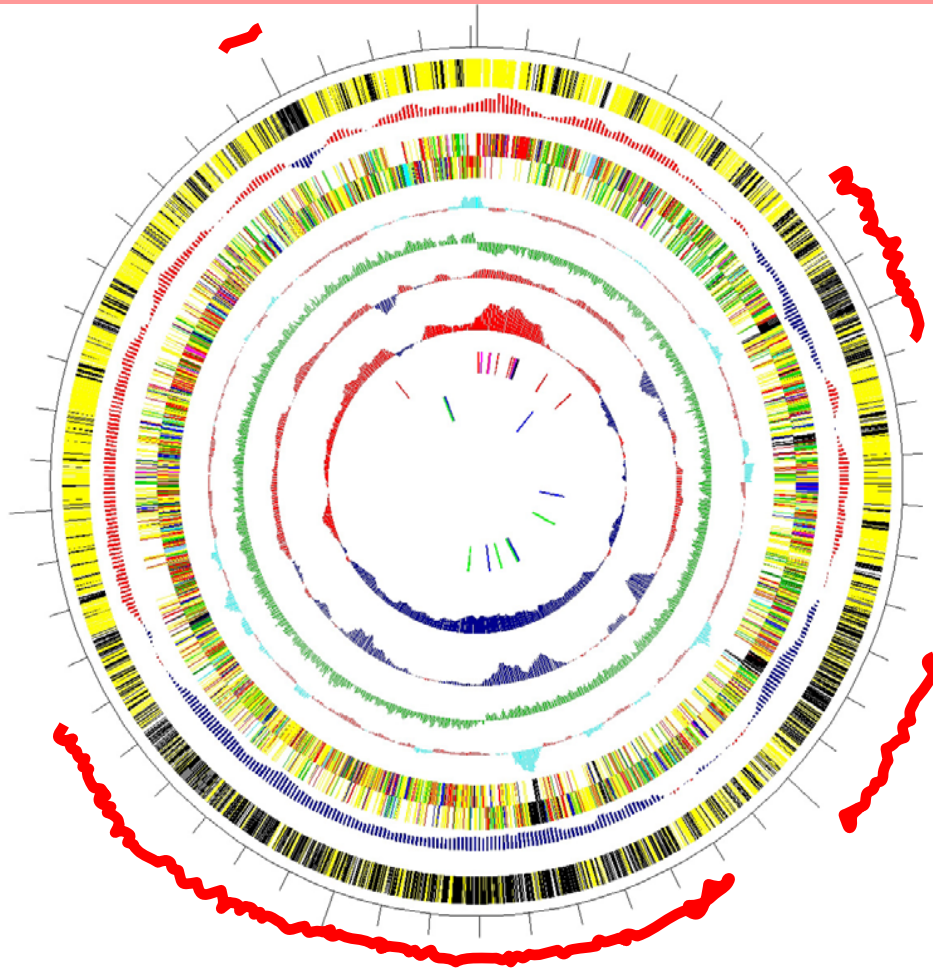


Whole-genome comparison of protein-coding regions of the strain *B. cereus* ATCC14579 (cluster T) to *B. anthracis* Ames (cluster C) and the strain NVH 391-98. Mean identities of orthologs are 93 and 80% respectively.



16S rRNA based phylogenetic tree for Bacilli, Streptococci and *S. aureus*. Divergence between the strain NVH391-98 (underlined in red) and strains of the *B. cereus* group is similar to that between *B. subtilis* and *B. licheniformis* (underlined in yellow).

B. cereus 14579 vs *B. cytotoxis* NVH391-98



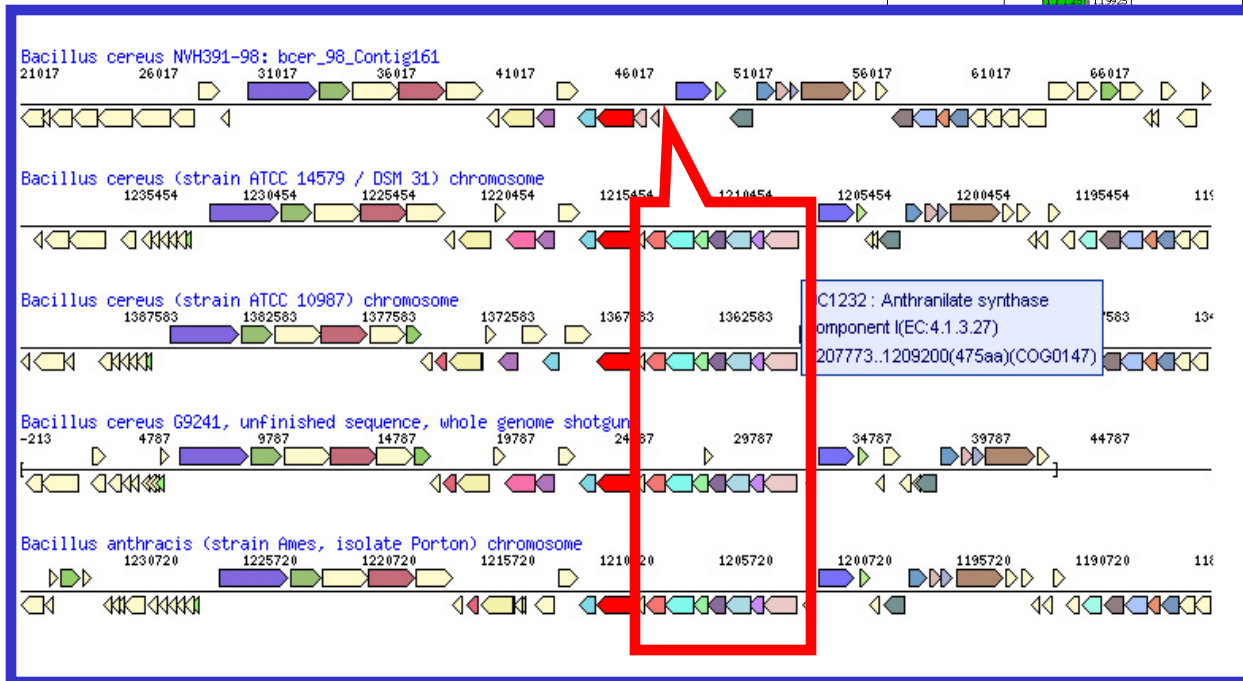
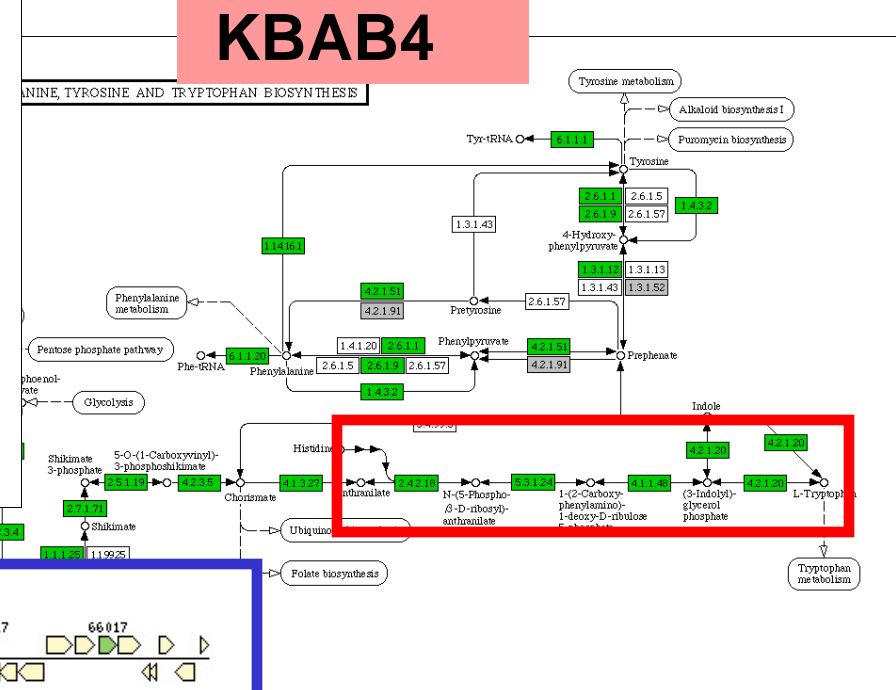
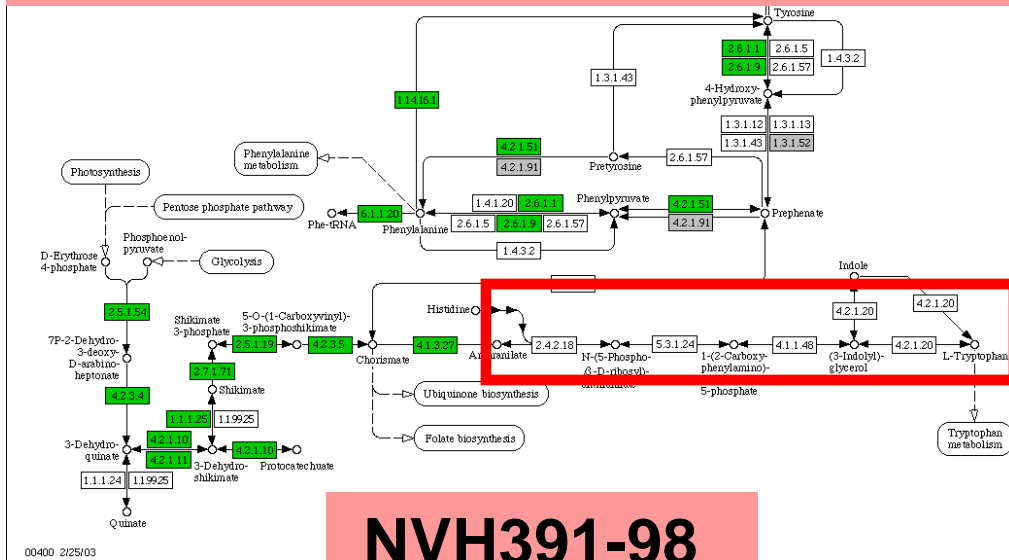
Circular representation of the chromosomal map of *B. cereus* ATCC14579. The first inner circle after the scale represents CDS present (yellow) or absent (black) in NVH 391-98. Red stretches outside highlight the regions absent in NVH391-98 compared to ATCC14579. Other internal circles represent distributions of different properties over the 14579 genome. Note the correlation of deletions in the NVH391-98 genome with location of pro-phages in the 14579 genome (black bars in the 7th and 8th circle from the center).



B. cereus 14579 chromosome size = 5412 kb
NVH391-98 chromosome size = 4082 kb

Linear representation of comparison between chromosomal map of *B. cereus* ATCC14579 and NVH 391-98. Contigs, assembled after random sequencing of NVH391-98 genome, aligned by using MUMmer (Delcher et al, Nucl Acids Res, 2002, 30, 2478-83) over the 14579 chromosome. Regions marked by red boxes are absent in the NVH 391-98 genome.

No trp byosynthesis in NVH391-98



Surprisingly, the tryptophan biosynthesis operon was found to be deleted in the strain NVH 391-98. Trp auxotrophy can therefore be one of the distinctive phynotypic characteristics of these new species of Bacilli. The relation of such auxotrophy to the pathogenesis of this strain is not clear. Another distinctive feature of the strain is the inability to grow at low temperatures (below 20°C, data not shown).

Current project status (07.03.2006)

NVH391-98

contigs > 1000000 bp : contigs = 2; bases = 4072474
contigs > 500000 bp : contigs = 2; bases = 4072474
contigs > 300000 bp : contigs = 2; bases = 4072474
contigs > 200000 bp : contigs = 2; bases = 4072474
contigs > 100000 bp : contigs = 2; bases = 4072474
contigs > 50000 bp : contigs = 2; bases = 4072474
contigs > 30000 bp : contigs = 2; bases = 4072474
contigs > 10000 bp : contigs = 2; bases = 4072474
contigs > 5000 bp : contigs = 2; bases = 4072474
contigs > 3000 bp : contigs = 4; bases = 4080383
contigs > 2000 bp : contigs = 6; bases = 4085724
contigs > 1000 bp : contigs = 6; bases = 4085724
contigs > 500 bp : contigs = 6; bases = 4085724
contigs > 1 bp : contigs = 9; bases = 4086341

projNVH.0.2026.fas length is 2752406
projNVH.0.73.fas length is 1320068
projNVH.0.1994.fas length is 4308
projNVH.0.7.fas length is 3601
projNVH.0.8.fas length is 2746
projNVH.0.13.fas length is 2595

Only contigs longer 2000 bp are listed. The contigs in red are of foreign origin (not related to the project 's organism).

KBAB4

contigs > 1000000 bp : contigs = 2; bases = 2574891
contigs > 500000 bp : contigs = 4; bases = 4133897
contigs > 300000 bp : contigs = 7; bases = 5338340
contigs > 200000 bp : contigs = 8; bases = 5567750
contigs > 100000 bp : contigs = 8; bases = 5567750
contigs > 50000 bp : contigs = 10; bases = 5694239
contigs > 30000 bp : contigs = 12; bases = 5769973
contigs > 10000 bp : contigs = 16; bases = 5844818
contigs > 5000 bp : contigs = 16; bases = 5844818
contigs > 3000 bp : contigs = 18; bases = 5853770
contigs > 2000 bp : contigs = 19; bases = 5856264
contigs > 1000 bp : contigs = 19; bases = 5856264
contigs > 500 bp : contigs = 21; bases = 5857430
contigs > 1 bp : contigs = 22; bases = 5857577

projAB4.0.232.fas length is 1459530
projAB4.0.1003.fas length is 1115361
projAB4.0.984.fas length is 915578
projAB4.0.927.fas length is 643428
projAB4.0.699.fas length is 418053
projAB4.0.850.fas length is 401395
projAB4.0.22.fas length is 384995
projAB4.0.38.fas length is 229410
projAB4.0.42.fas length is 75870
projAB4.0.40.fas length is 50619
projAB4.0.27.fas length is 44830
projAB4.0.17.fas length is 30904
projAB4.0.23.fas length is 26509
projAB4.0.21.fas length is 25727
projAB4.0.3.fas length is 12431
projAB4.0.4.fas length is 10178
projAB4.0.6.fas length is 4506
projAB4.0.7.fas length is 4446
projAB4.0.15.fas length is 2494

F0837/76

contigs > 1000000 bp : contigs = 2; bases = 2783230
contigs > 500000 bp : contigs = 4; bases = 4252342
contigs > 300000 bp : contigs = 5; bases = 4656958
contigs > 200000 bp : contigs = 6; bases = 4873467
contigs > 100000 bp : contigs = 8; bases = 5156071
contigs > 50000 bp : contigs = 8; bases = 5156071
contigs > 30000 bp : contigs = 9; bases = 5191416
contigs > 10000 bp : contigs = 14; bases = 5265546
contigs > 5000 bp : contigs = 15; bases = 5273575
contigs > 3000 bp : contigs = 16; bases = 5276724
contigs > 2000 bp : contigs = 19; bases = 5283438
contigs > 1000 bp : contigs = 25; bases = 5292627
contigs > 500 bp : contigs = 49; bases = 5311411
contigs > 1 bp : contigs = 55; bases = 5312863

projBC837.0.39.fas length is 1498742
projBC837.0.49.fas length is 1284488
projBC837.0.1060.fas length is 755721
projBC837.0.273.fas length is 713391
projBC837.0.1646.fas length is 404616
projBC837.0.53.fas length is 216509
projBC837.0.267.fas length is 162150
projBC837.0.338.fas length is 120454
projBC837.0.1673.fas length is 35345
projBC837.0.339.fas length is 18709
projBC837.0.5.fas length is 16924
projBC837.0.8.fas length is 15684
projBC837.0.100.fas length is 12151
projBC837.0.99.fas length is 10662
projBC837.0.24.fas length is 8029
projBC837.0.103.fas length is 3149
projBC837.0.36.fas length is 2338
projBC837.0.105.fas length is 2222
projBC837.0.40.fas length is 2154

- This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231 and Los Alamos National Laboratory under contract No. DE-AC52-06NA25396